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Strong Antimicrobial Acvitity of Lactic Acid Bacteria Isolated from Raw Goat Milk in Medan, North Sumatera, Against *Staphylococcus aureus*

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Abstract

Biopreservation is a method of foods preservation using natural ingredients by inhibiting the growth of spoilage food bacteria. Lactic acid bacteria is a bacteria that utilize as food preservation because it produce antimicrobial agent including bacteriocin and also act as probiotic. LAB could isolated from various natural source including raw milk. The aim of the study was to determined the antimicrobial activity of lactic acid bacteria (LAB) isolated from raw goat milk against food spoilage bacteria including Escherichia coli, Staphylococcus aureus, and Salmonella typhi. The result of the research indicated that the LAB isolated from raw goat milk(isolate EH1, EH2, EH3, EH4, EH5 and EH6) were Gram positive, homofermentative and negative in catalase test. The antimicrobial activity of LAB against indicator bacteria showed the strong antimicrobial activity against Staphylococcus aureus. The inhibition zone of EH1, EH2, EH3, EH4, EH5 and EH6 against S.aureus respectively were 19 mm ; 17,4 mm; 21,7 mm; 14,3 mm; 14,2 mm and 16 mm. The LAB isolates could not inhibit the growth of E.coli and S.typhi. It concluded that LAB isolated from raw goat milk has a potential as source of effective probiotic against Gram positive bacteria.

Keywords: Lactic Acid Bacteria (LAB); raw goat milk; antimicrobial activity; food spoilage bacteria.

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1. Introduction

Lactic acid bacteria (LAB) is a group of Gram-positive bacteria, cocci or rod shaped, not forming spores and produce lactic acid as final product of carbohydrate fermentation [1]. These bacteria are found abundant in nature as in milk, meat and various fermented foods as the dominant micro flora. LAB produces a variety antimicrobial agents including organic acids, hydrogen peroxide, diacetyl and a bactericidal protein, bacteriocin [2]. These substances have ability to inhibit the growth of pathogenic bacteria in gastrointestinal tract and food spoilage bacteria in food [3]. Lactic acid bacteria is an important bacteria in the process of food fermentation and beverages, including wide variety of dairy product, fermented vegetables, cheese, pickles and others [4]. One of the source of lactic acid bacteria are raw milk. Raw milk is a appropriate growth medium for microorganism. The quality and composition of microflora in raw milk is determined by many factors including the level of hygienicity of the production milk processing environment, temperatures and also microflora in the surrounding air [5]. Raw goat milk is reported to have a higher digestibility and lower allergy risk if than cow milk, in addition goat milk has short cain faaty acids and higher content of zinc, iron and magnesium. This fact led to that goat milk is appropriated medium for probiotics and prebiotics [6]. The aim of the study was to evaluate the anti microbial activity of lactic acid bacteria isolated from raw goat milk against pathogenic bacteria.

2. Experimental section

Materials

Materials used in this study are raw goat milk, MRS broth, MRS agar, pepton water, distilled water, hydrogen peroxide, crystal violet, ethanol, fuchsin, iodine, and crystal violet.

Instrumentation

Instrument used in this study are autoclave, laminar air flow cabinet, petri dishes, test tube, incubator, microscope, water bath, vortex, analytical, colony counters, etc.

Procedure

Total colony of Lactic Acid Bacteria

As many as 100 mL of raw goat milk obtained from local goat breeders in Medan, North Sumatera. The milk is stored in a sterile glass bottle and immediately taken to laboratory for analysis. The enrichment process are conducted by mixing as many as 1 mL raw samples mixed with 9 mL sterile MRS broth in test tube and incubated in 37°C at anaerobic condition. After incubation the serial dilution are conducted. 0,1 mL samples are mixed with 0,9 mL sterile pepton water in microtube 1,5 mL then homogenized by turning the microtube up and down. This is first dilution. The serial dilution continued until sixth dilution. As many as 0,1 mL from the last dilution are were spread on to the surfaces of MRS agar and then incubated in incubator at 37°C for 48 hours. After 48 hours the colony of LAB then counted using colony counter.

Biochemical characteristic

Six colonies from total colony of LAB was randomly chosen by repeated streaking onto MRS agar, namely EH1, EH2, EH3, EH4, EH5 and EH6. The biochemical characteristic were investigated for each isolate : Gram staining, catalase test and fermentation type.

Antimicrobial activity of LAB against pathogenic bacteria

Antimicrobial activity of each isolate against pathogenic bacteria (*E.coli*, *S.aureus*, and *S.typhi*) were investigated using disc diffusion assay. 1 loopful of each isolate were transferred into 9mL sterile MRS broth in test tube and incubated anaerobically. The pathogen bacteria (*E.coli*, *S.aureus* and *S. typhi*) were transferred into 9 mL sterile nutrient broth.. The incubation of LAB and pathogenic bacteria was conducted at 37°C for 18-24 hours. After incubation, the pathogenic bacteria were spread onto surfaces of nutrient agar using sterile cotton swab. The isolate of LAB were centrifuged at 6000 rpm for 20 minutes to obtained supernatant The blank discs (diameter 5 mm) were dipped into supernatant and then plated on the surface of nutrient agar. After 24 incubation, the inhibition zone were determined using calipers.

3. Result and discussion

LAB isolation and enumeration biochemical characteristic

The total colony of LAB from 1 mL raw goat milk was 30 x 10^6 CFU/mL. The growth of LAB from raw goat milk on the surfaces of MRS agar are shown in Figure 1.

Figure 1: The growth of LAB isolated from raw goat milk on MRS agar

The biochemical characteristic of each isolate were shown in table.1

Table 1: biochemical characteristic of LAB isolated from raw goat milk

LAB isolates	Biochemical characteristic		
	Gram staining	Fermentation type	Catalase test
EH1	positive	homofermentative	Negative
EH2	positive	homofermentative	Negative
EH3	positive	homofermentative	Negative
EH4	positive	homofermentative	Negative
EH5	positive	homofermentative	Negative
EH6	positive	homofermentative	Negative

The similar result was reported by Devi and his colleagues [7], which reported that lactic acid bacteria isolated from yoghurt gave the blue purple color with staining, hence all of the isolates were Gram positive. The catalase test of the isolates gave negative result. Lactic acid bacteria generally non respiratory and lack of catalase. Goyal and his colleagues [8] reported that lactic acid bacteria isolated from curd that collected from Gurgaoi (Hayana) and Laksmangarh (Rajastan) majority were Gram positive rods and cooci shaped and negative in catalase. From 28 isolates only 7 were not able to produce bubbles when mixed with hydrogen peroxide 3%. It indicated that lactic acid bacteria are absence of catalase enzyme.

Antimicrobial activity of lactic acid bacteria

The selected isolate of LAB were determined for their antimicrobial activity against pathogenic bacteria, *E.coli, S.aureus,* dan *S.typhi* using disc diffusion agar method. The evaluation of antimicrobial activity was conducted triplicate. Diamater of inhibition zone of LAB isolates were shown in table 2.

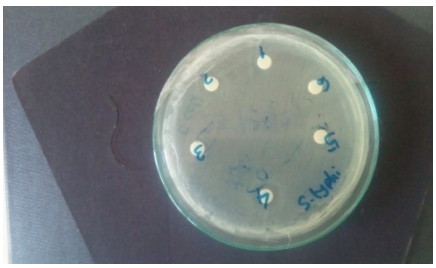
Table 2: inhibition zone of LAB against pathogenic bacteria

LAB isolates	Inhibition zone (mm)		
	S.aureus	*E.coli*	*S.typhi*
EH1	20,5	-	-
EH2	16,6	-	-
EH3	19,7	-	-
EH4	18,2	-	-
EH5	15,2	-	-
EH6	9,5	-	-

Table 2 showed that supernatant of LAB isolates only effective to inhibit *S.aureus*, where EH1 and EH3 showed

the strongest antimicrobial activity. The antimicrobial activity of LAB against pathogen bacteria showed in figure 2.

(a) (b)



(c)

Figure 2: The antimicrobial activity of LAB against pathogenic bacteria; (a) *S.aureus*, (b) *E.coli*, (c) *S.typhi*

One of antimicrobial agents of LAB is bacteriocin. Bacteriocin is the most potent bacterial growth inhibitor. Although this bacteriocin can be produced by a wide spectrum of bacteria, bacteriocin produced by LAB has a great interest because LAB have generally been recognized as safe (GRAS) [9]. Syukur and his colleagues [3] reported that Lab isolated from 'dadih' a traditional fermented milk product from West Sumatera Indonesia, showed the strongest antimicrobial activity against *S.aureus* and weak activity against *E.coli*. Bacteriocin are bioactive peptides which ribosomally synthesized by bacteria displaying antimicrobial activity against related or non related bacteria. These peptides are considered natural bippreservatives and their potential application in food industry has received great interest. Bacteriocin are active against Gram-positive pathogens such as *L.monocytogenes* and *S.aureus* and less effective against Gram-negative bacteria such as *E.coli*[10], because the cell wall of Gram positive bacteria allows passage of relatively large molecules and also bacteriocin.

4. Conclusion

From the result it can be concluded that raw goat milk have a high potential as a source of bacteriocin producing-lactic acid bacteria, however it has been proved that bacteriocin alone is not likely to ensure completely safety against Gram-negative bacteria such as *E.coli* and *S.typhi*.

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